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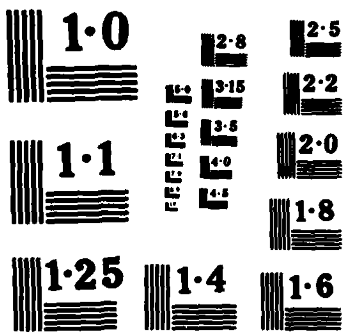

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Giant cells of characean algae were examined for electrophysiological sequelae to acute electromagnetic field irradiation at 10 mW/cm^2. Carrier frequencies ranged from 50 MHz to 12.4 GHz and a variety of modulation protocols were employed. No effects were detected at this power level.

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Submitted 08/16/85 by
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SYNOPSIS

SUMMARY OF ALL WORK ACCOMPLISHED:

This is provided in the sections following.

INDEX OF ALL TECHNICAL REPORTS:

This final technical report is the only technical report produced on this contract.

INDEX OF ALL PUBLICATIONS:

[1] K. M. Brunkard and W. F. Pickard, The membrane potential of characean cells exposed to amplitude-modulated, low-power 147-MHz radiation. Bioelectromagnetics 5, 353-356 (1984).

[2] A. V. Gokhale, K. M. Brunkard, and W. F. Pickard, Vacuolar hyperpolarizing offsets in characean cells exposed to mono- and bichromatic CW and to squarewave-modulated electromagnetic radiation in the band 200-1,000 MHz. Bioelectromagnetics 5, 357-360 (1984).

[3] A. V. Gokhale, K. M. Brunkard, and W. F. Pickard, The absence of significant short-term electromagnetic bioeffects in giant algal cells exposed to CW and pulse-modulated X-band bursts. IEEE Trans. Microwave Theory Techniques MTT-32, 795-797 (1984).

[4] A. V. Gokhale and W. F. Pickard, Evidence that characean membrane transport is not significantly altered by incident electromagnetic radiation. Radiation Res. 102, 300-306 (1985).

[5] A. V. Gokhale, W. F. Pickard, and K. M. Brunkard, Low-power 2.45-GHz microwave radiation affects neither the vacuolar potential nor the low frequency excess noise in single cells of characean algae. J. Microwave Power 20, 43-46 (1985).

[6] A. V. Gokhale and W. F. Pickard, Extremely low frequency resistance fluctuations of *Chara* membrane. Plant Physiol. To be published.

[7] W. F. Pickard, The electrical noise of a cell: Correlations between resistance noise and voltage noise. Submitted to Math. Biosci.

INTRODUCTION

The aims of this contract (N00014-82-K-0261) were (i) to use an extremely sensitive electrophysiological technique of W. F. Pickard and Y. H. Barsoum [J. Membrane Biol. 61, 39-54 (1981)] to screen for short-term electromagnetic bioeffects over frequency ranges of interest to the Office of Naval Research and (ii) to investigate irradiation modalities (e.g., mixed frequencies or pulse modulated fields) more characteristic of the shipboard environment than the single-frequency continuous-wave fields commonly employed in bioelectromagnetics research.

Not all of the seven papers listed above are of direct and obvious relevance to the these aims; and, in fact, some are peripheral. Hence, a brief guide the publications under the contract will be given before any details of the fulfillment of aims (i) and (ii) are provided. Paper [1] is listed only because it was carried out using equipment in part purchased under the contract and because it served as a test bed for a novel technique later employed in [4]; it will not be discussed further. Paper [2] employed power densities well above those which could reasonably be anticipated in a naval environment but is listed because it served to train A. V. Gokhale, who actually did most of the critical measurements under the contract, and because it employed novel frequency combining techniques; it will be discussed at slightly greater length later. Papers [3], [4], and [5] describe the studies intended to fulfill the aims of the contract; their contents will be excerpted later. Papers [6] and [7] arose from some surprising observations made in passing during work on paper [5]; they are of considerable relevance to membrane ion transport but will not be discussed further because their completion required very little time and because they are not directly related to the electromagnetic field sensitivity of an organism.

EXPERIMENTAL TECHNIQUES

Experimental technique has been exhaustively documented in papers [1] - [5]. Hence the description here will be limited to the briefest of overviews with emphasis placed upon the philosophy of the method.

If an electromagnetic field is to have an effect on a cell other than the directly thermal or the classically electrophysiological, then it must have a locus of action. One obvious locus is the cell membrane. Any action at this locus should exert some effect on the upon the electrophysiological properties of the cell, in particular upon the electrical potential of its interior but also upon its membrane resistance. There are many ways of searching for such effects, and several were tried during the course of this contract. What is of importance is that most are unambiguously quantifiable, several offer extremely high resolving power, and those of high resolving power tend in addition to be fast.

The exposure apparatus was a simple microstrip with a ceramic slab of dielectric constant 75 separating the strip from its subjacent ground plane; a channel, down which test solution was flowed, had been cut into the ceramic perpendicular to the strip. A giant cell of a characean alga (normally about 15 mm long and 300 μm in diameter) would be placed in the channel downsteam from the strip, impaled in its downstream end by a glass micropipette electrode (two electrodes for resistance measurements), and then slid upstream until its umimpaled end was under the strip. In this configuration, one end of the cell could be irradiated by the known quasi-TEM mode of the microstrip while the electrophysiological response of the cell was sensed nonperturbingly by the micropipette in its field-free downstream region. Moreover, since the cell impedance-matched the channel solution which in turn impedance-matched the ceramic slab, the electromagnetic field in the cell was known.

In the most common experiment, the cell was irradiated by a series of widely spaced bursts of radiation and the responses of its internal potential accumulated by a signal averager. Typically this internal potential was on the order of 50 mV, while a response on the order of 5 μV was detectable by signal averaging; this is a resolution of roughly 1 part in 10,000, and 5 μV is below the normal stochastic fluctuation of the cell's potential. In various experiments, there were employed bursts of CW, bursts of pulses, and bursts of swept frequency. Less commonly used techniques will be described briefly in the results sections below.

MIXED FREQUENCY STUDIES

The deck of a naval vessel is not a simple fixed-frequency electromagnetic environment. Many frequencies may be present at once, and some sources may hop their frequencies. The hypothesis that, at or below 10 mW/cm^2, a potpourri of frequencies could have a relevant biological effect even though any single one would not is unfalsifiable: for, however many combinations are shown to be of no effect, there will always be others which have not been tested. Hence, evaluation of this hypothesis can never be more than inadequate. Yet, if the envisioned effects of mixed frequencies are robust, then a random (exploratory) selection of frequency combinations might just reveal something positive. Acting on this possibility, three such combinations were assayed.

First [2], it was asked whether, at field levels well within the range where thermal effects are noted, the admixture of two discrete frequencies would reveal a nonlinear interaction that might be indicative of an athermal phenomenon. For this it was assumed that power windows were not a problem and that any positive findings would become monotonically more positive with increasing power levels. This possibility was examined using the burst irradiation technique at power levels roughly 10,000-fold the nominal 10 mW/cm^2 safe exposure level; at these power levels, clear thermal sequelae are detectable. Using mixtures of 245 & 810 MHz, of 366 & 705 MHz, or of 547 & 1019 MHz, no nonlinear interactions were detected.

Second [4], it was asked whether discrete bursts of X-band irradiation of non-constant frequency would have an effect. For this, the frequency was swept from 8.0 to 12.4 GHz either by a 50 kHz triangle wave or by a 20-50,000 Hz white noise source. At a variety of power levels from 0.01 to 500 mW/cm^2, no offset of cellular potential was detected by the burst irradiation technique.

Third [unpublished], preliminary measurements in which the irradiation was swept from 10-1,000 MHz by an audio-frequency triangle wave, revealed no effect at 10 mW/cm^2.

STUDIES OF EXPOSURE TO AMPLITUDE-MODULATED FIELDS

The electromagnetic fields to which naval personnel are exposed tend to more pulsed than CW. Therefore it is desirable to examine the hypothesis that, even though continuous wave irradiation might have no athermal sequelae, non-constant irradiation might. Once again, this hypothesis is not definitively falsifiable. However, if careful study of a variety of different non-constant fields turns up no athermal effects, then one can express guarded optimism that such effects are at worst subtle.

First [2], at the distinctly thermal power level of 145,000 mW/cm^2, the effect of bursts of CW was compared to the effect of bursts of the same carrier frequency 100% squarewave modulated at 100, 1000, or 10000 Hz. No statistically significant difference was observed although nine different carrier frequencies from 200 to 1000 MHz were tried.

Second [3], at 10 mW/cm^2 and nine different carrier frequecies from 8.2 to 12.4 GHz, three different burst irradiation protocols were employed: CW, 1.0 µs pulses 1 ms apart, and 0.1 µs pulses 0.1 ms apart. In no instance was an effect seen on the cellular potential despite the 1:10000 resolving power of the technique.

Third [3], at 9.09 GHz and power levels of 10 and 100 mW/cm^2, twenty different combinations of pulse interval and pulse width were employed. Again, the burst irradiation protocol revealed nothing.

Fourth [3], at 9.09 GHz, bursts of CW were compared with bursts of 1 µs pulses separated by 1 ms. Neither burst protocol revealed an effect at 0.1, 0.2, 0.5, 1, 2, 5, or 10 mW/cm^2.

Fifth [4], twenty different combinations of pulse period and pulse length were tried at power densities of 10 or 100 mW/cm^2. The burst protocol revealed no effect at carrier frequencies of 50, 147, 450, or 917 MHz.

Sixth [4], a 450 MHz carrier was modulated by 60 µs wide pulses. A lock-in amplifier examined the cellular potential for shifts correlated with the pulses. At a variety of pulse repetition frequencies from 4 to 64 Hz and power levels from 0.2 to 100 mW/cm^2, no effects were detected over 2 min averaging periods despite the fact that any offset above 250 nV should have been obvious.

STUDIES OF LONGER TERM IRRADIATION

Because the burst protocol is specialized for the examination of effects whose rise times are a second or less, it could well miss effects which require longer term irradiation to become manifest. Therefore, additional studies were undertaken employing 2 min or 1 h irradiation times.

First [4], the internal potential of the cell was dc-nulled and amplified 100-fold. This gave a trace in which irradiation-correlated offsets on the order of 250 μV would have been apparent if they occurred during a two minute period of irradiation or within a two minute control period following irradiation. At 10 mW/cm^2 and at frquencies of 50, 147, 450, and 917 MHz , continuous-wave irradiation had no effect.

Second [4], the amplified and nulled cellular potential was integrated to give a slope in which irradiation-correlated kinks would have bee apparent if they had occurred on a time scale of ten to sixty minutes and had arisen from long-term cell potential shifts of 5 mV or more. After a 1 h control period, continuous-wave irradiation at 10 mW/cm^2 was applied for 1 h and was followed by a further 1 h control period. There was no detectable effect at frequencies of 50, 147, 450, and 917 MHz .

Third [4], the amplified and nulled cellular potential was band-pass filtered (0.2 - 2.0 Hz), was RMS-to-dc converted, and was integrated to yield a slope in which irradiation-correlated kinks would have been apparent if they had occurred on a time scale of ten to sixty minutes and had arisen from shifts of 25% or more in the low frequency excess (voltage) noise of the cell. Under the same conditions of irradiation as in the previous test, no effect of irradiation was noted.

STUDIES AT 2450 MHZ

Because 2450 MHz is by a wide margin the most popular frequency for performing bioelectromagnetics screening experiments, it was singled out for special study [5].

At incident power densities of 10 mW/cm^2, no effect was discovered in studies with the burst irradiation protocol or with the 2 min or 1 h protocols of the previous section.

To explore whether environmental stress might potentiate microwave sensitivity, selected cells were examined in the above three fashions while being stressed by (i) the chloride channel blocker ethacrynic acid at a concentration of 100 µM, or (ii) a temperature of 15 °C (standard was 25 °C), or a temperature of 10 °C . The stressed cells showed no microwave response.

Finally, it was asked whether cells irradiated for 1 h by an incident fields of either 10 or 100 mW/cm^2 would show a significant change in membrane resistivity. (These experiments were carried out at 15 °C using the two pipette method of resistivity measurement.) No statistically significant changes in membrane resistivity were seen.

DISCUSSION AND CONCLUSIONS

Because neither naval personnel nor the general public are apt to encounter average power densities in excess of the old ANSI standard of 10 mW/cm^2, screening experiments which employ this level of incident irradiation are probably sufficient, at least in a first search for bioelectromagnetic phenomena. Our search for effects at this power density turned up nothing despite the large number of different frequencies, irradiation protocols, and electrophysiological end points used. If, in the giant cells of the Characeae, there is a cellular effect of acute (i.e., one hour or less) exposures, it must be presumed to be subtle -- unless of course the experimental studies were in some way flawed. Three possible flaws will be discussed.

First, the experimental technique may have been flawed. It is always possible that one or another of the several techniques used may have been flawed, either in design or execution; but it does seem rather unlikely that all were flawed. Moreover, the burst irradiation protocol did yield positive results at distinctly thermal levels of irradiation [2]. Because many other independently performed studies in this laboratory on the same experimental preparation likewise revealed no athermal effect despite the many other frequencies used (For a summary, see Reference [4].), it is doubtful that flawed technique can totally account for the absence of observed effect: probably either (i) there is no significant effect to observe or (ii) what effect there is is subtle.

Second, the experiments were carried out at known incident power densities rather than at known specific absorption rates. This is not the place to debate the philosophical question of what the most appropriate descriptor of electromagnetic exposure might be. Indeed, the answer to that question may be somewhat irrelevant because, despite the fact that by any reasonable measure a goodly amount of exposure was taking place (See [4] for SAR estimates.), no effects were seen at 10 mW/cm^2.

Third, parameter space is vast, and the experiments actually performed covered only an infinitesimal fraction of it. The hypotheses that there are sharp frequency resonances, or that there are sharp power resonances, or that the cell's environment was unsuitable can never be falsified. The experiments are flawed by inadequate coverage of parameter space, and the flaw is irremedial. But, any practical importance of this flaw only serves to substantiate the assertion that effects missed are indeed subtle because a large number of parameter combinations were tried in the unsuccessful search for athermal bioeffects.

In summation, the experiments performed under this contract revealed no biological effects of acute low-level electromagnetic irradiation of the experimental preparation used. They offer no evidence that such irradiation may be biologically deleterious.

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